
STUDY PROTOCOL – COVER PAGE

Official Title:

Acute Effects of Combined and Isolated Caffeine and Theanine Supplementation on Physical and Cognitive Performance in Competitive Athletes: A Randomized, Double-Blind, Placebo-Controlled Crossover Study

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Method

Study Design

This study employed a randomized, double-blind, placebo-controlled, crossover design to examine the effects of caffeine (CAF), theanine (THE), their combination (COM), and a placebo (CON) on physical and cognitive performance in young, healthy, competitive athletes. Each participant completed all four experimental conditions in a randomized order, with a washout period of at least 72 hours between sessions to minimize potential carryover effects. The randomization sequence was generated by an independent researcher using a computer-based randomization tool (Urbaniak & Plous, 2013). To ensure familiarity and minimize learning effects, all participants completed a familiarization session one week prior to the experimental trials. Physical and cognitive performance were assessed across multiple primary outcomes, including maximal strength, intermittent aerobic endurance, and eye-hand coordination. All procedures were conducted in accordance with the of Helsinki. The study protocol was approved by the Sinop University Human Research's Ethics Committee (Approval No: 2025/337), and all participants provided written informed consent prior to participation.

Participants

12 males and 8 females athletes (19.9 ± 2.4 years; 167.3 ± 7.6 cm height; 63.5 ± 10.2 kg body mass; 27.5 ± 6.7 kg lean body mass; 22.6 ± 8.2% body fat; 22.6 ± 2.3 $kg \cdot m^{-2}$ BMI) from both team and individual sports were recruited for the study (Table 1). During the first laboratory visit, anthropometric and body composition assessments were performed to characterize the participant sample. Standing height and body mass were measured to the nearest 0.1 cm and 0.1 kg using a calibrated stadiometer and digital scale (Seca, Hamburg, Germany). Body composition was determined via multi-frequency bioelectrical impedance analysis (InBody 770; InBody Co., Seoul, South Korea). All measurements were conducted in the morning, with participants wearing light clothing and having abstained from exercise, food, and caffeine for at least 3 hours prior to testing.

Inclusion criteria were: (i) active participation in individual or team sports at a competitive level, (ii) age between [insert range, e.g., 18-30] years, and (iii) absence of any known medical or metabolic disorder. Exclusion criteria included: (i) history of cardiovascular, neurological, or musculoskeletal disorders within the past 12 months, (ii) known intolerance or hypersensitivity to caffeine or theanine, (iii) allergy to mannitol or other excipients used in capsule formulation, (iv) current use of prescription medications, and (v) physician-directed restriction of caffeine or theanine consumption.

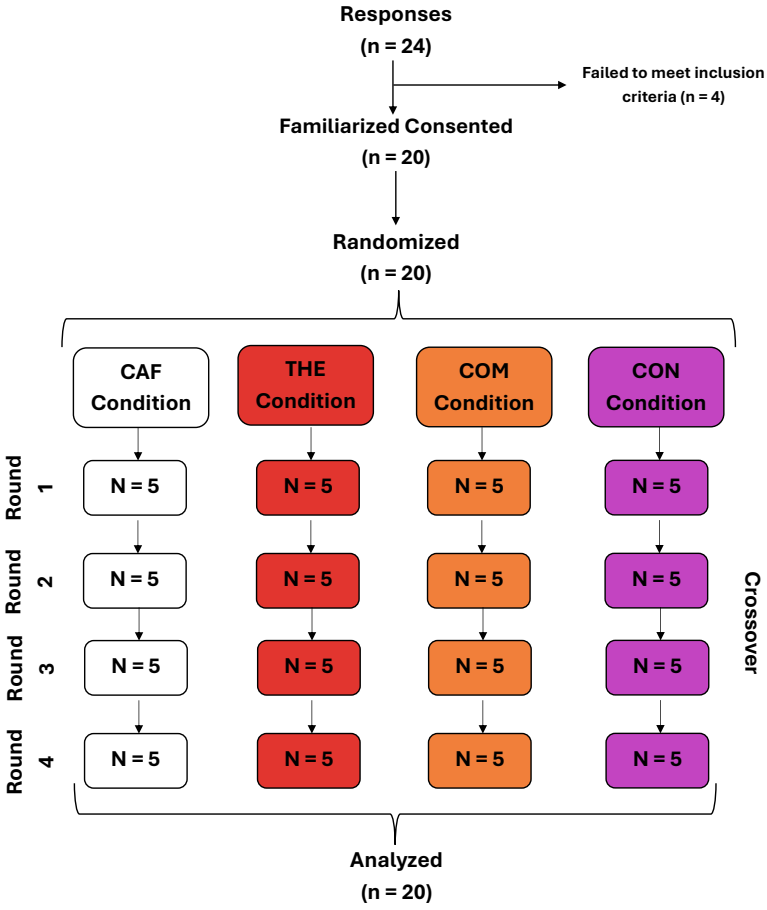

Figure 1. Flowchart

The required sample size was estimated using an a priori power analysis (G*Power 3.1.9.7; Düsseldorf, Germany) for a repeated-measures ANOVA with four conditions (CAF, THE, CAF + THE, CON), assuming a moderate effect size ($f = 0.25$), $\alpha = 0.05$, and statistical power $(1 - \beta) = 0.80$. The analysis indicated a minimum required sample of 16 participants. To account for potential attrition or missing data, 20 athletes were recruited; all completed the study protocol.

Supplementation Protocol

Capsules were ingested 60 minutes prior to testing to allow sufficient time for peak plasma caffeine concentrations (Pallares et al., 2013). The supplementation conditions were structured as follows: caffeine (CAF, 3 mg/kg^{-1} anhydrous caffeine), theanine (THE, 200 mg L-theanine), combined caffeine and theanine (COM, 3 mg/kg^{-1} caffeine + 200 mg theanine), and placebo (CON, mannitol). Caffeine capsules contained anhydrous caffeine powder (Oxford brand pure caffeine powder; ISO 14001 certified; 98.5% purity; The Oxford Vitality Health Company Ltd., London, UK), and theanine capsules contained pure L-theanine powder Ocean (L-Theanine, 200 mg; Orzax Pharmaceuticals, Istanbul, Turkey; active ingredient manufactured in Germany). Placebo capsules contained mannitol, a non-nutritive sugar alcohol with no known ergogenic or ergolytic properties (Flueck et al., 2016). All supplements were administered in visually identical gelatin capsules to ensure blinding across conditions. Each participant ingested the same number of capsules (five in total) in all conditions to maintain consistency in capsule count and total mass. Neither participants nor researchers could distinguish between conditions due to the capsules' identical appearance, texture, and color.

Participants were instructed to refrain from alcohol consumption and strenuous exercise for 24 hours before each trial and to abstain from any dietary supplements throughout the study period. They maintained 24-hour dietary records for the day preceding each test and a weekly log of their habitual caffeine intake. To standardize pre-trial nutritional status, participants replicated their recorded dietary intake and hydration patterns before each visit. To preserve ecological validity and reduce potential caffeine withdrawal effects, participants were permitted and encouraged to maintain their usual daily caffeine consumption during the study period, as recommended by Tallis et al. (2021) and Pickering & Kiely (2019).

Data Collection Tools

All sessions were conducted under standardized computer laboratory and indoor sports hall conditions. The eye-hand coordination assessment was conducted in a quiet, temperature-controlled laboratory using a desktop computer system. Ambient conditions were maintained at approximately 22 ± 1 °C and 50 ± 5% relative humidity. All other performance tests (strength and aerobic endurance) were carried out in an indoor sports hall under comparable conditions (temperature 21-23 °C, relative humidity 45–55%). Environmental parameters were monitored at the start of each session using a calibrated digital room thermo-hygrometer. Lighting, background noise, and testing times were standardized across all trials to ensure intra-individual consistency.

Testing sessions were held in the morning at 09:00 a.m. to minimize the potential effects of circadian variation and diurnal performance fluctuations. The sequence of tests was standardized as follows: eye-hand coordination, maximal strength, and aerobic endurance. Adequate rest intervals were provided between tests to prevent fatigue and carry-over effects. The following measurements were collected during each experimental condition:

Strength Tests

Maximal isometric strength was assessed using a Lafayette digital dynamometer (Takei Scientific Instruments Co. Ltd, Tokyo, Japan) to measure handgrip, back, and leg strength. The device was checked for calibration prior to each testing session according to the manufacturer's recommendations.

Participants performed three maximal voluntary contractions for each test, with at least 60 seconds of rest between attempts to minimize fatigue. Handgrip strength was assessed with participants seated, shoulders adducted, elbows flexed at 90°, and the dynamometer handle adjusted to individual hand size. Back strength was measured in a standing position with the participant pulling upward on a handle attached to the dynamometer's base platform while maintaining a neutral spine. Leg strength was evaluated in a similar posture, with participants exerting maximal upward force through a chain-linked platform while keeping their knees slightly flexed.

The mean of the three trials for each test was recorded as the participant's strength score, expressed in kilogram-force (kgf) as displayed by the device. All strength assessments were performed in accordance with the standardized procedures for isometric testing recommended by the American College of Sports Medicine (ACSM, 2021). American College of Sports Medicine. (2021). ACSM's Guidelines for Exercise Testing and Prescription (11th ed.). Wolters Kluwer.

Aerobic Endurance

Aerobic endurance was assessed using the Yo-Yo Intermittent Endurance Test Level 1 (Yo-Yo IET-1), which evaluates participants' ability to perform repeated bouts of exercise with brief recovery periods. The protocol followed the standardized procedures described by Bangsbo et al. (1996). Testing was performed on a flat, non-slip indoor surface with two parallel lines marked 20 m apart and a recovery line placed 5 m behind the starting line. Prior to testing, participants completed a standardized 5-minute dynamic warm-up consisting of jogging and mobility drills. Bangsbo, J. (1996).

Participants ran back and forth between the 20-m lines in time with audio cues from the official Yo-Yo IET Level 1 recording, with running speed progressively increasing throughout the test. After each 2 × 20 m shuttle, a 5-second active recovery period was provided, during which participants jogged to and from the recovery line. The test was terminated when a participant failed to reach the 20-m line on two consecutive occasions, and the total distance covered (m) was recorded to the nearest meter as the performance outcome.

Eye-Hand Coordination

Eye-Hand Coordination Test (Multidirectional and Unpredictable Direction) was used to evaluate participants' ability to perform accurate, continuous manual motor actions based on dynamic visual processing. The test was administered in a quiet, temperature-controlled laboratory (22 ± 1 °C; relative humidity 50 ± 5%) using CogniFit's proprietary digital platform (CogniFit Inc., New York, USA) on a desktop computer.

They were instructed to remain seated comfortably, maintain a neutral wrist position, and use their dominant hand for all trials. During testing, participants tracked a small moving circle ("ball") that followed an unpredictable multidirectional trajectory. The task required maintaining a cursor (crosshair) within the moving circle as precisely as possible. Visual feedback was provided continuously: green when on-target, orange when near the boundary, and red when the cursor left the target area. Each assessment included two initial learning segments (7 s slow + 7 s fast) followed by twelve testing segments alternating between slow (≈5 cm/s) and fast (≈10 cm/s) movement speeds, each lasting either 2333 ms (short) or 7000 ms (long), for a total duration of approximately 5-7 minutes including instructions. Prior to the main test, participants completed a brief familiarization session that included standardized on-screen practice trials lasting approximately 1 minute to minimize learning effects. Optional 10-15 second rest intervals were allowed between trials to avoid fatigue.

The following performance metrics were recorded automatically by the CogniFit software:

Accuracy (%): Percentage of time the cursor remained inside the circle (0–100; higher = better).

Accuracy - Fast Speed (%): Accuracy during fast segments (≥ 10 cm/s).

Accuracy - Slow Speed (%): Accuracy during slow segments (≤ 5 cm/s).

Accuracy - Long Segments (%): Accuracy during 7000 ms segments (sustained tracking).

Accuracy - Short Segments (%): Accuracy during 2333 ms segments (brief tracking).

Distance from Circle Center (pixels): Mean pixel distance between cursor and target center (0–1200; lower = better precision)

Performance scores were automatically normalized against CogniFit's age- and sex-matched normative database (percentile 0–100), following the developer's validity index ranges and completion-time criteria (54–58 s valid window). All assessments adhered to the manufacturer's standardized task protocol and validity thresholds (CogniFit Inc., 2023).

Statistical Analysis

The normality of the data set was initially evaluated using the Shapiro-Wilk test. Subsequently, the assumption of sphericity was examined through Mauchly's test, with the Greenhouse-Geisser correction applied in cases where sphericity was violated. Differences in strength, aerobic endurance, and eye–hand coordination outcomes across the four supplementation conditions (CAF, THE, COM, and CON) were analysed using a one-way repeated-measures analysis of variance (ANOVA). When significant main effects were observed, Bonferroni-adjusted pairwise comparisons were conducted to identify specific between-condition differences. The magnitude of effects was quantified using partial eta squared (ηp^2), interpreted as small (0.10–0.24), moderate (0.25–0.39), or large (≥0.40). For pairwise comparisons, Cohen's d for repeated measures and corresponding 95% confidence intervals (CI95%) were reported and interpreted as trivial (<0.20), small (0.20–0.49), moderate (0.50–0.79), or large (≥0.80) (Cohen, 1992). All statistical analyses were performed using IBM SPSS Statistics version 30.0 (IBM Corp., Armonk, NY, USA) and statistical significance was accepted at $\alpha = 0.05$ (two-tailed). Graphical visualizations were generated with GraphPad Prism version 10.0 (GraphPad Software, San Diego, CA, USA).